resistant strains (MIC 256 mg/L). The moderately erythromycin-resistant strains were also resistant to tetracycline and hybridized with erm7853 and with tetQ probes.

Conclusion: To our knowledge, this is the first time that the *erm7853* and the *tetQ* related genes have been observed in *C. difficile* strains. These results provide additional evidence of transfer of antibiotic resistance genes among intestinal bacteria.

Beta-lactamase/ESBL

P80 Extended-spectrum ß-lactamases (ESBLs) in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*

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Objectives: Determination of frequencies of *E. coli* and *K. pneumoniae* strains which produce ESBLs, in isolates from hospitalized patients, and evaluation of susceptibility of the strains to selected antibiotics.

Methods: In the first trimester of 1998, 137 *E. coli* strains and 52 *K. pneumoniae* strains were isolated from patients in four hospitals in Poznan, Poland. The strains were identified using ID 32 GN tests and the ATB system. ESBL-producing strains were detected using the double-disk test with clavulanic acid as ß-lactamase inhibitor. Strain susceptibility to selected antibiotics was determined using the disk diffusion technique, as specified by the NCCLS.

Results: Three *E. coli* strains (2.2%) and 21 *K. pneumoniae* strains (40.4%) were producing ESBLs. All the ESBL-producing strains were also resistant to aminoglycoside antibiotics and to doxycycline. High sensitivity of ESBL-producing *E. coli* strains (100%) and *K. pneumoniae* strains (over 70%) to fluoroqinolones was demonstrated. One strain of *K. pneumoniae* was resistant to imipenem.

Conclusion: Application of imipenem in treatment of *E. coli* and/or *K. pneumoniae* infections should be restricted to strains sensitive to the antibiotic, which produce ESBLs.

P81 Occurrence of extended-spectrum beta-lactamases and inducible beta-lactamases in clinical strains of Gram-negative rods

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Objectives: To check the actual situation concerning the occurrence of Gram-negative rods producing extended-spectrum ß-lactamases (ESBL)s and inducible ß-lactamases (IBLs) in clinical specimens from newborns hospitalized in the Department of Neonatology in Cracow. In addition, 1187 cases of neonates with infectional risk factors were retrospectively estimated in relation to the frequency of the systemic infections caused by Enterobacteriaceae clinical isolates resistant to third-generation cephalosporins. Such determinations have not been performed in this hospital before.

Methods: The strains were identified in automatic ATB system using strips with biochemical tests. ESBL-producing strains were detected with the double disk diffusion test according to Jarlier et al. Inducible ß-lactamases were determined using the double disk method according to Sanders and Sanders.

Results: Over 2 years (1997–98), 360 strains belonging to the Enterobacteriacae family (64% of all strains) and non-fermenting rods (36%) were isolated. New groups of Gram-negative bacteria (mainly *Enterobacter cloacae* 8% and *Klebsiella pneumoniae* 86%) resistant to the majority of commonly used ß-lactam antibiotics were found. In total, 19% ESBL-producing strains and 30.5% strains with IBL activity were detected. In a study performed in the years 1993–97 in our hospital, 56% of *Klebsiella pneumoniae* isolates were producing ESBLs, but data obtained for particular years were dramatically different, varying between 11% and over 83% in 1997. A substantial increase in thenumber of IB-producing strains of *Enterobacter cloace* was observed in the years 1993–96 (from 58% to 91.6%).

Conclusions: The obtained results confirm the necessity of continuous and reliable monitoring of ESBL- and IBL-producing strains among Gram-negative rods isolated from clinical materials.

P82 Accumulation of norfloxacin (NOR-Ac) in extended-spectrum ß-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kpn) deficient or not in porins

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Objectives: To evaluate NOR-Ac in ESBL-Kpn and the effect of the energy inhibitor cyanide *m*-chlorophenylhydrazone (CCCP) in this accumulation, and to study the relationship between NOR-Ac, porin expression, and MICs of NOR.

Methods: Twenty-seven clinical isolates of ESBL-Kpn were studied. MICs of NOR were determined by microdilution (NCCLS guidelines). Strains for which the MIC of NOR was ≥2 mg/L were considered resistant (NOR-R). Porin expression was determined by SDS-PAGE of purified outer membranes. ESBL production was detected by a reduction of the MIC of ceftazidime and/or cefotaxime by clavulanic acid. Duplicated tubes of cells were preincubated with NOR (10 mg/L). One set of tubes were subsequently treated with CCCP (0.1 mM). Extracellular NOR was eliminated by centrifugation through a silicon oil barrier. NOR-Ac was measured by spectrofluorometry.

Results: Fourteen strains produced porins (7 NOR-R, 7 NOR-S), and 13 strains were porin deficient (8 NOR-R, 5 NOR-S). Ranges of NOR-Ac were 141±70 to 225±44 for 11 strains (type A), and 427±93 to 490±88 for 16 strains (type B). CCCP determined increased NOR-Ac in all type A strains (NOR-Ac after CCCP treatment: 331±32 to 435±64). CCCP did not affect NOR-Ac in type B strains. Type A strains included 4 NOR-S and 7 NOR-R strains. Ten of 11 type A strains were porin deficient, while 13 of 16 type B strains expressed porins.

Conclusions: Most ESBL-Kpn strains deficient in porins show decreased NOR-Ac caused by an energy-dependent mechanism. This mechanism was observed in both NOR-susceptible and NOR-resistant strains.

P83 Relationship between ß-lactamase (BLS) production and outer membrane protein (OMP) profiles and activity of carbapenems against *Acinetobacter baumannii*

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Objectives: To investigate the relationship between imipenem (IMP) and meropenem (MPM) activity and BLS production and OMP profiles in *Acinetobacter baumammii* (AB).

Methods: Twenty-six AB strains, representing antibiotypes and pulsotypes of organisms isolated from blood during 1995–97, were

studied. Strains were biotyped according to Bouvet and Grimont. MICs of ticarcillin (TIC), ceftazidime (CAZ), IMP and MPM, alone or combined with clavulanic acid (CA) or BRL42715 (BRL), were determined by microdilution. OMPs were separated by SDS-PAGE. PIs of BLS were determined by isoelectric focusing (pH 3–9). Hydrolysis of IMP was determined by spectrophotometry and by a microbiological assay.

Results: Five biotypes were identified: 9 (n=9), 6 (n=8), 2 (n=6), 18 (n=2) and 11 (n=1). MIC_{90}s (mg/L) were: 512 (TIC, TIC=CA, CAZ, CAZ=CA), 128 (TIC=BRL, CAZ=BRL), 16 (IMP, IMP=CA), 8 (MPM, MPM=CA), 4 (MPM=BRL) and 0.5 (IMP=BRL). Four OMP profiles were observed: A (n=19), B (n=5), C (n=1) and D (n=1). Four out of five isolates with profile B (characterized by the absence of a 25-kDa OMP) were resistant to carbapenems (MICs IMP and MPM=8 mg/L). All isolates had BLS with pIs of either 9 or 8.3. Thirteen isolates additionally had a BLS of pI 6.3, and in some cases other BLS (pIs: 6.8 and 5.4). Hydrolysis of IMP (mU/mg protein) in all 13 isolates containing the BLS 6.3 was higher (0.1–0.7) than in isolates lacking the 6.3 BLS (not detectable to 0.06). MICs (mg/L) of IMP and MPM for isolates containing the 6.3 BLS ranged from 4 to 32, and for isolates lacking the 6.3 BLS from 0.06 to 2.

Conclusions: Resistance of AB to IMP (but not MPM) is reverted by BRL. Decreased activity of carbapenems in AB is associated with a BLS of pI 6.3 and in some cases with the loss of a 25-kDa OMP.

P84 Metallo-ß-lactamases in *Pseudomonas aeruginosa*: a new problem for Europe

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A multidrug-resistant *Pseudomonas aeruginosa* strain (VR 143/97) with unusually high-level resistance to imipenem (MIC 256 mg/L) was isolated from a surgical wound of a patient admitted to the intensive care unit of the Verona University Hospital. A crude cell-free extract prepared from this strain exhibited imipenem- hydrolyzing activity, which was almost completely inhibited by treatment with EDTA and was restored by addition of Zn^{2+} to the EDTA-treated sample. A bla_{IMP}-specific probe did not hybridize to strain VR 143/97 in a colony blot assay, indicating that a metallo-carbapenemase other than IMP-1 was produced by this strain.

From March 1997 to February 1998, a further 9 isolates of *P. aeruginosa* showing high-level resistance to imipenem (MICs 128–256 mg/L) were cultured in samples of patients from different wards of the same hospital. All strains were resistant to piperacillin, piperacillin/tazobactam, ticarcillin/clavulanate, ceftazidime, cefepime, imipenem, and meropenem.

The DNA fingerprinting patterns of *Xba*I-digested total DNA preparation by PFGE for the 9 isolates suggest that 8 of the 9 strains were clonally related to the index strain. Cloning and sequencing of the gene encoding the new metallo-carbapenemase (bla_{IMP2}) was undertaken and primers derived from the sequence were used in amplification reactions of strains' DNA. PCR analysis confirmed the presence of bla_{IMP2} in all strains, including the non-genetically related strain. This suggested that the gene was apparently transferable, at least among *P. aeruginosa* strains.

The nosocomial outbreak we describe, preceded by the isolation of a multidrug- resistant carbapenemase-producing *P. aeruginosa* strain in the UK, confirms the emergence of new carbapenemases outside Japan and also emphasizes the urgent need for early recognition of strains producing these enzymes.

P85 Extended-spectrum-ß-lactamase-producing *K. pneumoniae* outbreak in a cardiopediatric ICU

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Introduction: An extended-spectrum-ß-lactamase-producing *K. pneumoniae* (ESBL-Kp) outbreak (October 1997 to March 1998) was detected in the cardiopediatric ICU from our hospital.

Materials and methods: Screening for colonization/infection was performed with 1 mg/L ceftazidime MacConkey agar plates. MICs for different ß-lactam and aminoglycoside antibiotics were determined according to the NCCLS guidelines. Transconjugants (nalidixic acid- and kanamycin-resistant *E. coli* MC4100) and transformants (*E. coli* TG-1) were obtained. ß-Lactamase isoelectric focusing was performed with the PhastSystem (Pharmacia). Bla_{TEM} primers were used on plasmid DNA of the transformants to amplify the ß-lactamase gene. To assess the relatedness between the ESBL-Kp isolates, random amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) were performed.

Results and conclusions: Colonization/infection with the ESBL-Kp isolate was detected in 10 patients. Microbiology screening, patient isolation, personnel handwashing measures and changes in antibiotic use limited the outbreak. The susceptibility profiles (range MICs, mg/L) of the ESBL-Kp isolates were as follows: ceftazidime, 16–64; cefotaxime, 32–128; aztreonam, 16–32; co-amoxiclav, 4/2–8/4; cefoxitin, 4–8; imipenem, <=0.25; gentamicin, 8; tobramicin, 8; and amikacin, <=4. Oxi-imino-ß-lactam and aminoglycoside resistances were co-transferred by conjugation and transformation. A single TEM-type ß-lactamase band of 5.9 was observed in the ESBL-Kp transconjugants and transformants. RAPD and PFGE demonstrated similar patterns in all the ESBL-Kp isolates.

P86 Post-ß-lactamase inhibition effect (PLIE) of clavulanic acid against ß-lactamase-producing strains of *Klebsiella pneumoniae* and *Haemophilus influenzae*

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The PLIE could be one among many factors explaining why the ß-lactamin/ß-lactamase inhibitor combinations remain effective in vivo after serum concentrations of inhibitor have fallen below levels which are active in vitro. The purpose of this study was to investigate and characterize the in vitro PLIE of clavulanic acid (CA) against ß-lactamase-producing strains: *K. pneumoniae* (MIC values: amoxycillin (AMX) 256 mg/L; CA 64 mg/L; AMX-CA 4 mg/L) and *H. influenzae* (MIC values: AMX 32 mg/L; CA 32 mg/L; AMX-CA 1 mg/L).

A stationary-phase inoculum of about 10^7 of each bacterium served as a CA=1 mg/L controller or was pre-exposed for 2 h to either CA alone or CA plus AMX at various concentrations: *K. pneumoniae* CA 2 or 4 mg/L, AMX 4.8 or 16 mg/L; *H. influenzae* CA 0,5, 1 or 2 mg/L, AMX 1, 4, 8 or 16 mg/L. The dilution needed to remove the ß-lactamase inhibitor or both drugs was 10^{-2} or 10^{-3} according to the strain The hourly bacterial counts following the removal of drugs were carried out on solid supplemented agar media. Control cultures were exposed to AMX alone after dilution and showed a regrowth delay (RD), possibly due to the time needed by

bacteria to synthesize ß-lactamases in sufficient amounts after dilution. Control experiments allowed us to clearly differentiate PLIE from postantibiotic effect (PAE) or from RD.

RD values ranged from 0.5 h to 1.5 h for *K. pneumoniae, and* from 0 h to 13 h for *H. influenza*. PAE=0 for *K. pneumoniae*, but the values for *H. influenza* ranged from 1 h to 5 h. PLIE vales for *K. pneumoniae* ranged from 0 h to 5 h, while the values for *H. influenza* ranged from 0 h to 15 h These data, differing from previously published results, suggest the existence of an authentic PLIE different from RD and PAE, as proved by results obtained after the pre-exposure to CA alone.

P87 Antimicrobial susceptibility of *Escherichia coli* (Ec) and *Klebsiella pneumoniae* (Kp) clinical isolates producing extended-spectrum beta-lactamases (ESBLs) in a pediatric hospital in Greece

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Objectives: To evaluate the emergence of Ec and Kp isolates producing ESBLs in various wards in our hospital and their in vitro susceptibility to antimicrobials over a 1-year period (November 1997 to October 1998).

Methods: Thirty-eight Ec and 74 Kp non-repetitive strains producing ESBLs, isolated from 98 patients, were tested by the disk diffusion method to 18 antimicrobials according to NCCLS recommendations. MICs were determined using a commercially available microdilution system (Sensititre, West Sussex, UK). ESBLs were detected by the double-disk synergy test (DDST).

Results: Of ESBL producers, 75% were recovered at neonatal and oncology units, while 3.5% were from PICU patients. MIC_{50} and MIC_{90} values (mg/L) of cefotaxime (Cx), ceftazidime (Caz), cefepime (Fep), amikacin (Ak), gentamicin (Gn), tobramycin (Tb), piperacillin/tazobactam (Tzp), imipenem (Imp) and ciprofloxacin (Cip) were as follows:

ciprofloxacin (Cip) were as follows:

		Cx	Caz	Fep	Ak	Gn	Tb	Tzp	Imp	Cip
Ec	MIC_{50}	≤4	>16	0.5	16	4	>8	≤2/4	≤0.25	≤0.06
	MIC_{90}	32	>16	4	32	>8	>8	8/4	≤0.25	≤0.06
Kp	MIC_{50}	≤4	>16	1	8	2	8	≤2/4	≤0.25	≤0.06
	MIC_{90}	16	>16	4	16	>8	>8	16/4	≤0.25	≤0.06

Conclusions: DDST is necessary, since ESBL producers often have low-level resistance to third-generation cephalosporins and may be mis-identified. Antimicrobial agents, such as Fep, Imp, Cip and Tzp, remain extremely active.

P88 Detection of extended-spectrum beta-lactamase (ESBL)-producing strains at the Military Medical Academy, Sofia, Bulgaria

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Objectives: This study presents data on the prevalence of ESBL-producing strains (65 of family Enterobacteriaceae) at MMA-Sofia for a 9-month period and compares two methods of detection.

Methods: The microorganisms were investigated by the Mini API system (BioMerieux, France). The double-disk (DD) test and ATB-BLSE (BioMerieux, France) were used. The ATB-BLSE method shows the recovery by sulbactam determining the MIC of antibiotic (aztreonam and ceftazidime) on its own and in the presence of an inhibitor.

Results: The 44 ESBL-producing strains were recovered from: endotracheal aspirates (16), urine (13), surgery wounds (11), blood (2), and bile (2). *Klebsiella pneumoniae* is prominent among all ESBL-positive isolates (90.7%). The most ESBL-positive isolates originated from the ICU (47.7%) and surgery clinics (25%).

Conclusions: During the 36-week period, 4.2% of all isolates produced ESBLs. The DD method is useful and less expensive. The ATB-BLSE method (first time in our country) provided more sensitivity, automated reading and epidemiologic analysis. This test is useful for selecting strains for future molecular analysis.

P89 Antimicrobial susceptibilities and ß-lactamase content of *Yersinia* strains isolated from aquatic environments

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Objectives: Studies related to ß-lactamase content of the previously named '*Y.enterocolitica*-like' species and in particular of *Yersinia intermedia* have not appeared in the literature. We report susceptibilities and ß-lactamase contents of *Y. enterocolitica* and *Y. intermedia* isolated in aquatic environments of Greece.

Methods: Thirty-seven *Yersinia* spp. strains (seven *Y. enterocolitica* and 30 *Y. intermedia*) isolated from various aquatic environments were biotyped, serotyped and tested for their susceptibility. Screening for induction and inhibition of ß-lactamase expression was performed by double-disk tests, and ß-lactamase content was analyzed by isoelectric focusing.

Results: On the basis of combined biotyping/serotyping results, *Y. enterocolitica* isolates were scattered in five and *Y. intermedia* in 17 groups. Various patterns of ß-lactam insensitivity were detected, including ampicillin and ticarcillin (35 isolates), cephalothin (33), carbenicillin (32), amoxycillin/clavulanate (23), and cefoxitin (22). No correlation between biotype or serotype and the susceptibility pattern of the isolates was apparent. In all cases two distinct ß-lactamase bands were detected, presumably a penicillinase and a cephalosporinase, with pls varying from 9 to 9.5 for the former and from 5.5 to 7.8 for the latter. Accordingly, by disk diffusion synergism tests, both inducible cephalosporinase activity against third-generation cephalosporins and inhibition of resistance to penicillins were detected in all *Y. enterocolitica* and *Y. intermedia* isolates.

Conclusions: The above data indicate that two ß-lactamases, analogous to those previously described in clinical strains of *Y. enterocolitica*, are also present in *Yersinia* spp. from environmental sources; variations in susceptibility profiles occur, probably due to quantitative differences in their production.

P90 Detection of extended-spectrum ß-lactamases (ESBLs) among enterobacteria: comparison of the Vitek ESBL and double-disk tests

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Objectives: There is great interest in developing tests for the detection of ESBL-producing enterobacteria. The Vitek ESBL test has been proven a reliable indicator for the presence of ESBLs among *E. coli* and *Klebsiella pneumoniae* strains. In our region, the presence of ESBLs among enterobacteria is very high and there are indications

of their spread among species other than *E. coli* and *K. pneumoniae*. In this respect, we evaluated the ability of the Vitek ESBL test to detect ESBLs in comparison with the double-disk test.

Methods: Standard Vitek ESBL and double-disk tests were performed on 228 enterobacterial isolates from clinical specimens in hospitals of Thessaloniki, Greece. In cases of discrepant results, both tests were repeated. Strains with discrepant results after repeat testing were analyzed for ß-lactamase production after conjugal transfer of resistance.

Results: Of the 228 strains examined (77 *E. coli*, 75 *K. pneumoniae*, 33 *Enterobacter cloacae*, 7 *E. aerogenes* and 36 other enterobacteria), 68 strains (22 *E. coli*, 43 *K. pneumoniae*, 2 *E. cloacae* and one *Serratia marscecens*), were found to produce ESBLs by both tests. In five cases (4 *E. clocae* and one *E. aerogenes*), the double-disk test was positive, while the Vitek ESBL test failed to detect ESBLs. Further analysis by conjugal transfer, hydrolytic activity and analytic isoelectric focusing showed that these strains possessed ESBLs.

Conclusions: It seems, that in some instances, derepressed AmpC ß-lactamases in our *Enterobacter* strains mask detection of ESBLs by the Vitek ESBL test.

P91 *Serratia marcescens* in a tertiary care hospital—study over 2 years

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Objectives: To evaluate the frequency of *Serratia marcescens* isolation in comparison to other nosocomial pathogens during a 2-year period.

Methods: Our study was performed in a 1201-bed tertiary care hospital from 1995 to 1996. We reviewed our laboratory records to assess the frequency of *S. marcescens* infection and/or colonization in different wards and compare it to other nosocomial pathogens—*S. marcescens* isolates from 22 ICU patients in 1996 were genotyped by RAPD-PCR.

Results: In 1996 we observed a twofold increase in isolation of *S. marcescens* compared to 1995: 276 and 140 respectively.

	ICU	Surgery-9 units	General Medicine-8 units	Other units-6 units
1995	100	75	47	18
1996	245	135	121	20

The most frequent sites of isolation were: respiratory specimens (224), drains (196), urine (13), blood (77) and wounds (69). The rate of *S. marcescens* isolation from blood in 1996 (3.4%) equaled the rates for *Pseudomonas aeruginosa* (3.3%) and *Acinetobacter baumannii* (3.6%) but was still lower than those for *Escherichia coli* (4.6%) and *Staphylococcus aureus* (8.5%). PCR genotyping revealed the persistence of one predominant strain responsible for infections in 18 ICU patients in 1996.

Conclusions: During the studied period, *S. marcescens* became one of the most frequent nosocomial pathogens in our hospital. This was probably due to the spread of endemic clones as a result of cross-contamination and transfer of colonized patients between units. Infections caused by *S. marcescens* were difficult to eradicate despite targeted antimicrobial therapy which might have contributed to increased serratia isolations.

P92 In vitro activity of piperacillin/tazobactam against *Klebsiella pneumoniae* clinical isolates, producers or not of extended-spectrum beta-lactamases

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The aim of this study was to determine the in vitro activity of piperacillin/tazobactam against 81 clinical isolates of *K. pneumoniae*. The clinical specimens received at the Microbiology Department in the Hospital Universitario de la Princesa were processed according to standard microbiological procedures, and 81 *K. pneumoniae* isolates were identified by MicroScan Panels following the manufacturers' recommendations. A double-disk diffusion method was applied to detect extended-spectrum beta-lactamases (43 isolates were positive and 38 were negative). Minimum inhibitory concentrations (MICs) were determined by an agar dilution technique using Mueller–Hinton agar. The inoculum was applied with a Steer replicator and plates were incubated at 37°C for 18 h. The following antibiotics were studied: piperacillin/tazobactan (P/T), amoxycillin/clavulanic acid (AMC), cefotaxime (CTX), ceftriaxone (CRO), cefepime (CPI), imipenem (IMP) and meropenem (MER).

Results: The MIC_{90}s were 16/4 mg/L for P/T, 16/8 for AMC, 16 for CRO, 16 for CTX, 4 for CPI, 0.25 for IMP and 0.032 for MER in ESBL-positive strains, and 4/4 mg/L for P/T, 8/4 for AMC, 0.064 for CRO, 0.125 for CTX, 0.125 for CPI, 0.125 for IMP and 0.016 for MER in ESBL-negative strains. The percentage of susceptibility was 25.6% for AMC, 81.4% for CTX, 83.8% for CRO, 93.1% for P/T, 97.7% for CPI and 100% for IMP and MER in ESBL-producing *K. pneumoniae*, and 92.1% for AMC and 100% for CTX, CRO, CPI, IMP, MER and P/T in ESBL non-producer *K. pneumoniae*.

Conclusions: All beta-lactams showed excellent in vitro activity against ESBL-producing *K. pneumoniae*. Moreover, piperacillin/tazobactam, cefepime and both carbapenems showed good in vitro activity against EBSL-producing *K. pneumoniae*.

P93 Antimicrobial susceptibility to beta-lactams and beta-lactamases in *Acinetobacter baumannii*

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Objective: To study the antimicrobial susceptibility to beta-lactams (BLM) and the production of beta-lactamases (BLS) in *Acinetobacter baumannii* clinical isolates.

Methods: One hundred and fifty-six strains from different clinical samples were studied. The MICs of ampicillin (AMP), ticarcillin (TIC), piperacillin (PIP), ampicillin–sulbactam (AS), amoxycillin–clavulanic acid (AMC), ticarcillin–clavulanic acid (TICCL), piperacillin–tazobactam (TZP), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CEF), imipenem (IMP) and meropenem (MEP) were determined by an agar dilution method according to NCCLS recommendations. BLS production was studied by nitrocefin hydrolysis in crude sonic extracts, and pI was determined by isoelectric focusing (polyacrylamide gels, 3.5–9.5). Inhibitory profiles of BLS to clavulanic acid (CLAV), cloxacillin (CLOXA), sulbactam (SULB) and aztreonam (AZT) were determined by a spectrophotometric assay.

Results: A low antimicrobial susceptibility to most BLM was observed: AMP 1.9%; TIC 19.8%; PIP 10.2%; AS 84.6%; AMC 14.1%; TICCL 33.9%; TZP 37.8%; CTX 8.9%; CAZ 15.3%; CEF 17.3%; IMP 88.4%; MEP 89.1%. The association of BLM with inhibitors did not increase the susceptibility. Most strains were BLS

positive (92.9%). A chromosomic cephalosporinase (Cse), with pI 8, inhibited by CLOXA and AZT but not by CLAV and SULB, was demonstrated in 81.4% of the strains. Cse=TEM-1 (pI 5.4) was demonstrated in only one strain. A BLS with pI 7.7 not inhibited by inhibitors was shown by 10.2% of isolates. This percentage corresponded to strains with high-level resistance to carbapenems. One strain with low-level resistance to carbapenems showed a different BLS with pI 6.8, also not inhibited by inhibitors.

Conclusions: Chromosomic cephalosporinases predominate in these *A. baumannii* isolates and are correlated with low antimicrobial susceptibility to beta-lactams. The resistance to carbapenems is due to two novel BLS which confer different degrees of resistance to carbapenems.

P94 Description of two beta-lactamases in carbapenem-resistant *Acinetobacter baumanni* strains

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Objectives: To describe two novel beta-lactamases (BL) in carbapenem-resistant *Acinetobacter baumannii* (CRAb) clinical isolates that were not epidemiologically related.

Methods: Two strains of CRAb (no. 38 and no. 113) were studied. The MICs of imipenem (IMP), meropenem (MEP), cefotaxime (CTX), ceftazidime (CAZ), amoxycillin–clavulanic acid (AMC), ticarcillin (TIC), ticarcillin–clavulanic acid (TICCL), piperacillin (PIP), piperacillin–tazobactam (TZP) and ampicillin–sulbactam (AS) were determined by an agar dilution method according to NCCLS recommendations. The isoelectric point (pI) was studied on crude sonic extracts by isoelectric focusing (polyacrylamide gels, 3.5–9.5). Inhibitory profiles of BL to clavulanic acid (CLAV), cloxacillin (CLOXA), sulbactam (SULB), aztreonam (AZT) and EDTA were determined by the nitrocefin assay. The specific enzymatic activity of benzylpenicillin and cefaloridine was determined by a spectrophotometric analysis, and the hydrolysis of CTX, CAZ, carbenicillin (CARB), oxacillin (OXA), IMP and MEP was studied by a microbiological assay.

Results: The results of MIC determinations (mg/L) in strains no. 38 and no. 113 were, respectively: IMP 16, 128; MEP 8, 128; CTX 64, 128; CAZ 16, 128; AMC 128, 128; TIC 512, 512; TICCL 1024, 1024; PIP 128, 256; TZP 16, 256; AS 2, 8. BL of both strains showed respectively a pI of 6.8–7 and 7.7. The percentages of inhibition were, respectively: CLAV 3, 7; CLOXA 18.7, 10.1; AZT 12.4, 9.5; SULB 14, 14.8; EDTA 12 and 13.2. Both BL showed a predominant penicillinase activity. The hydrolysis of IMP, MEP, CARB and OXA was demonstrated in both BL, and the hydrolysis of CTX was observed in BL of strain no. 113.

Conclusions: Two novel 'serin-beta-lactamases' are implicated in the resistance to carbapenems in *A. baumannii*. These enzymes are penicillinases with similar biochemical characteristics, but they differ in the level of resistance to carbapenems and cephalosporins.

P95 Transfer of ceftazidime and cefotaxime resistance from multiresistant hemolytic *Escherichia coli* producing extended-spectrum beta-lactamases

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Objectives: The strain of hemolytic *Escherichia coli* was studied during a systematic survey of production and transferability of extended-spectrum beta-lactamase (ESBL) in ceftazidime-resistant Enterobacteriaceae.

Methods: *E. coli* No. 151 was isolated in Dérer's University Hospital in Bratislava. Antimicrobial susceptibility testing was performed by the disk diffusion test. The relative rate of hydrolysis of antibiotics was estimated by the macro-iodometric method. ESBL profiling of strains was performed using the double disk diffusion test. Transfer of genes for antibiotic resistance was performed in mixed liquid cultures. Cells of the donor and recipient strain were mixed in nutrient broth and the mixture was incubated overnight and then plated on agar with rifampicin plus one of the antibiotics present in the resistance spectrum of the donor strain. Transconjugant colonies were recorded after overnight incubation. As recipient strains we used *E. coli* K-12 No. 3110 rif+ and *Proteus mirabilis* P-38 rif+.

Results: *E. coli* No. 151 was resistant to the following beta-lactams—carbenicillin, cephalotin, cephazolin, cefuroxime, cefotaxime, ceftazidime and aztreonam—and directly transferred to both recipient strains the resistance to carbenicillin, cephalotin, cefotaxime, ceftazidime and aztreonam. The relative rate of hydrolysis of cefotaxime, ceftazidime and aztreonam in *E. coli* No. 151 was inhibited after the addition of clavulanate almost to zero values. The double-disk diffusion test demonstrated the presence of ESBL hydrolyzing cefotaxime and aztreonam in the original strain *E. coli* No. 151 and also the presence of ESBLs hydrolyzing cefotaxime, ceftazidime and aztreonam in transconjugant clones of *E. coli* K-12 or *Proteus mirabilis* P-38 recipient strains.

Conclusions: Production of extended-spectrum beta-lactamases in *E. coli* No. 151 and their conjugative transfer to susceptible recipient strains shows the possibility of spread of antibiotic resistance in clinically important Enterobacteriaceae in the hospital environment.

P96 In vitro activity of ceftazidime, cefepime and aztreonam against extended-spectrum beta-lactamase-producing nosocomial *Klebsiella pneumoniae*

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Objectives: To evaluate the in vitro activity of ceftazidime, cefepime and aztreonam in ESBL-producing *K. pneumoniae* isolates from various hospitals.

Methods: MICs were determined by an agar dilution method with 2-fold dilutions of antibiotics. To visualize the presence of ESBL in strains, the double-disk diffusion test was used.

Results: From 50 *K. pneumoniae* isolates, 24 were isolated in Hospital Pribram, Czech Republic, and 26 in different hospitals in Bratislava. MICs of 50 *Klebsiella pneumoniae* isolates were as follows:

MIC (mg/L)							
4	8	16	32	64	128	256	
Ceftazidime (*n*)	5	3	3	7	7	3	22

Cefepime (*n*) 15 11 8 2 5 3 6
Aztreonam (*n*) 4 4 1 5 4 5 27
n=number of isolates

Production of ESBLs was positive in 41 of these isolates in various combinations of inhibition of beta-lactamases by clavulanic acid (cla). In 41 cases production of beta-lactamase hydrolyzing cefotaxime was indicated, hydrolysis of aztreonam was demonstrated in 28 isolates, in 4 isolates production of beta-lactamase hydrolyzing ceftazidime was indicated, and 33 isolates showed production of ESBL hydrolyzing cefepime, despite the susceptibility of some isolates to this drug. The most frequent pattern of ESBL production, i.e. three zones of inhibition (cla/cefotaxime, cla/cefepime and cla/aztreonam) was observed in 20 isolates.

Conclusions: From three compared beta-lactams, cefepime was the most effective antibiotic against *K. pneumoniae* isolates studied.

P97 Several outbreaks caused by ESBL-producing *Enterobacteriaceae* strains in a Warsaw hospital

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Objectives: To characterize ESBL-producing Enterobacteriaceae in a large hospital extensively using expanded-spectrum cephalosporins.

Methods: Thirty-five isolates belonging to seven different Enterobacteriaceae species were collected as all ESBL producers over a period of 4 months (1996–97) in a hospital in Warsaw. MICs of beta-lactams and aminoglycosides were evaluated by the agar dilution method. Isolates were typed by RAPD and subjected to mating experiments. Beta-lactamase content was studied by IEF; ESBL activity was detected by the bioassay approach. ESBL families were identified by PCR. Plasmid DNA was purified from a representative group of isolates and subjected to fingerprinting analysis.

Results: Two different types of ESBL were identified in the analyzed isolates. One of these, with a pI of 8.4, has determined a specific resistance phenotype with MICs of cefotaxime much higher than those of ceftazidime. The 8.4 beta-lactamases belonged to the CTX-M family of ESBLs and most probably were the CTX-M-3 enzyme. They were produced by 27 isolates of all identified species. The *blaCTX-M* genes were located on very similar large conjugative plasmids conferring also resistance to aminoglycosides. The high prevalence of *CTX-M* producers in the hospital has resulted from both plasmid dissemination and clonal spread of several strains of different species. SHV enzymes with a pI of 8.2 represented the other ESBL type. These were produced by non-related *Klebsiella pneumoniae* and *E. coli* strains carrying plasmids of various fingerprints. These strains could have emerged by independent selection or have been introduced from other hospitals.

Conclusions: A high consumption of broad-spectrum cephalosporins together with lack of ESBL monitoring procedures before 1996 have resulted in a complex epidemiology of ESBL-producing microorganisms in Polish hospitals.

P98 In vitro activity of cefotetan against enterobacteria with extended-spectrum beta-lactamases

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Objectives: Extended-spectrum beta-lactamases (ESBLs) are an increasing cause of resistance to third-generation cephalosporins in Enterobacteriaceae, especially klebsiellae and *Proteus mirabilis*. The aim of the present study was to determine the in vitro activity of cefotetan against ESBL-producing clinical isolates.

Methods: The activity of cefotetan was assessed against 140 recent clinical isolates of enterobacteria collected during a multicenter survey in Italy. The presence of an ESBL was tested by the double-disk potentiation method and subsequently confirmed. The minimal inhibitory concentrations (MICs) were determined by the agar dilution method for cefotetan and 13 other antimicrobial agents, including cefoxitin, cefepime, ceftriaxone, ceftazidime, imipenem, meropenem, amikacin and ciprofloxacin. On selected strains the influence of inoculum size (10^5 versus 10^7 CFU/spot), the minimal bactericidal concentrations and the time-kill curves were also determined.

Results: Cefotetan was highly effective against the strains tested, exerting a rapid bactericidal effect. Its MIC_{50} and MIC_{90} were 0.5 and 2 mg/L respectively, its activity being superior to that of cefoxitin (4–32 mg/L), comparable to that of imipenem (0.5–4 mg/L) and weaker only than that of meropenem (0.06–0.25 mg/L). For the strains studied, a high rate of cross-resistance was observed between extended-spectrum cephalosporins, gentamicin (51.8%) and ciprofloxacin (40.3%).

Conclusions: Cefotetan had excellent activity against ESBL-producing Enterobacteriaceae and may be clinically useful in treating infections caused by these organisms.

P99 Antibiotic susceptibility of 64 *Stenotrophomonas maltophilia* strains determined by Etest and the agar dilution method according to ß-lactamase production and ß-lactam phenotype

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This study evaluated the susceptibility of 64 *Stenotrophomonas maltophilia* strains to ticarcillin (TIC), ticarcillin–clavulanate (TIM), imipenem (IMP), ceftazidime (CAZ), latamoxef (MOX), tobramycin (TOB), ciprofloxacin (CIP), tetracycline (TET) and rifampin (RIF) by Etest and conventional agar dilution methods. Plates were read after 24 h of culture at 37°C. ß-Lactam phenotypes were deduced from susceptibility to TIC, CAZ, MOX, IMP and aztreonam with and without clavulanic acid and ß-lactamase production (isofocalization). When results were analyzed as percentages of strains susceptible at the breakpoint, 80–90% agreement between the methods was observed. However, when MIC ratios were analyzed, correlations were poor, with 41% (TIM), 72% (CAZ) and 91% (CIP) of Etest MIC results within ±2 dilutions of the reference agar dilution. Among ß-lactam antibiotics, ticarcillin–clavulanate was found to be the most active by the two methods with 75% of susceptible strains. It was less active on L1 and/or L2 high-level ß-lactamase-producing strains. MIC distributions were slightly different for the two methods for most antibiotics and depended on ß-lactam phenotype and/or ß-lactam induction. Due to the great variability of ß-lactamase expression, the choice of susceptibility method for *S. maltophilia* deserves further study.

P100 Cloning and sequencing of the gene encoding a novel Oxa-type beta-lactamase isolated from *Aeromonas caviae*

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An *Aeromonas caviae* (AC) strain (P239), which presented a beta-lactam phenotype characterized by resistance to ampicillin, ticarcillin and cephalothin (MICs 64 mg/L), was studied for cloning of the gene responsible for beta-lactamase production. After chromosomal DNA preparation, two genes encoding a typical cephalosporinase enzyme and a new Oxa-type beta-lactamase were cloned into pCLL2300 (respectively 2.5 and 1.8-kb insert) and expressed into *E. coli* JM109. This later beta-lactamase (Oxa-Cav) had a pI of 8.5, in the same range (8–9, PAGE isofocusing) as *A. sobria* and *A. hydrophila* oxacillinase-producing strains. Oxa-Cav enzyme from the cloned gene was well expressed and hydrolyzed beta-lactam antibiotics such as oxacillin, ampicillin, ticarcillin, cephalothin and aztreonam but not cephalothin or cefotaxime. Beta-lactamase activity was inhibited by clavulanic acid. The nucleotide sequence of the gene was found to be only 70% homologous to the sequence of Oxa-12 (*A. jandei*) and ampS (*A. sobria*) previously described. The deduced protein sequence, which contained amino acid motifs common to other plasmid or chromosomal-mediated class D beta-lactamases, revealed 76% identity with Oxa-12, 74% with ampS and less than 40% with other class D beta-lactamases. As previously suggested, this report confirms that *Aeromonas caviae* may produce at least two beta-lactamases. Oxacillinase-type beta-lactamase appears to be ubiquitous in *Aeromonas* species.

P101 Extended spectrum beta-lactamases (ESBLs) in clinical isolates of Enterobacteriaceae

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Objectives: To study the prevalence of ESBLs in clinical isolates of Enterobacteriaceae isolated during 1997.

Methods: Seventy-one isolates of *Escherichia coli*, 69 *Klebsiella pneumoniae*, 34 *Serratia marcescens*, 31 *Enterobacter* spp., 30 *Proteus mirabilis*, 21 *Citrobacter* spp., 16 *Providencia* spp. and 10 *Klebsiella oxytoca* were tested for production of ESBLs by double-disk synergy test performed with ceftriaxone, cefotaxine, ceftazidime and amoxycillin–clavulanate disks.

Results: ESBL production was observed in 8.4% *Escherichia coli*, 59.4% *Klebsiella pneumoniae*, 44.1% *Serratia marcescens*, 25.8% *Enterobacter* spp., 20% *Proteus mirabilis*, 28.6% *Citrobacter* spp., 6.2% *Providencia* spp. and 20% *Klebsiella oxytoca* isolates; 57% ESBL producers would have been reported to be susceptible to ceftazidime and 61% to cefotaxime according to the routine susceptibility method.

Conclusions: Our results confirm the need for reliable tests in the detection of ESBL-producing strains.

P102 Multiresistant *Klebsiella pneumoniae* (extended-spectrum beta-lactamase positive) in the intensive care untit

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Objectives: To describe the impact of extended-spectrum beta-lactamase (ESBL)-positive, multiresistant *Klebsiella pneumoniae* (MRKP) in the intensive care unit (ICU), and typing of strains.

Methods and results: In 21 ICU patients an ESBL-positive MRKP was isolated during a period of 4 months. Resistance to gentamicin was found in 20 of the 21 strains. Results of RAPD, plasmid restriction fragment analysis and integron PCR of these and other isolates point to: (1) a cluster of identical isolates from 20 ICU patients; (2) a second type from one ICU patient; (3) a third unrelated type. Among the infections caused by MRKP were pneumonia, (uro)sepsis, brainabces, meningitis, and infected prostheses. The MRKP was found in surgical patients mainly (multi-trauma, neurosurgery, aneurysm aorta operations). Most patients were ventilated (14/20). The period of ventilation of patients with MRKP was 27 days (without MRKP 7 days). The MRKP caused serious, complicated infections in 8 patients (5 died), while 12 patients had either carriage or an easy-to-treat infection. The MRKP caused failure of initial antibiotic treatment and failure of selective decontamination (SD) of the digestive tract in ventilated patients.

Conclusions: Risk factors for MRKP were major surgery, ventilation, SD of the digestive tract, and third-generation cephalosporins. Barrier precautions stopped the spread of MRKP.

P103 Comparison of screening methods for detection of extended-spectrum ß-lactamases (ESBLs)

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Objectives: To compare the expediency and cost–benefit ratio of the Etest and double-disk synergy test (DDT) in the diagnosis of ESBL producers among clinical isolates.

Methods: 3637 Gram-negative rods isolated from various materials from patients hospitalized in 12 different clinics in the period April 1997 to June 1998 were tested by: the disk-diffusion method for antibiotic susceptibility (NCCLS), DDT (amoxycillin clavulanic acid versus aztreonam, cefotaxime, ceftazidime and ceftriaxone) for ESBL and E-test (AB Biodisk, Sweden) for ESBL. ESBL production was first detected by DDT in 192 (5.3%) of 3637 examined strains, and then DDT-positive results of 100 selected strains were counterchecked with Etest strips containing ceftazidime 1 clavulanic acid combination (TZ 1 TZL). ESBL (+) MIC TZ TZL 4.

Results: In the group of 100 strains ESBL positive by DDT, Etest was positive in 82, negative in 3 and inconclusive in 15. Resistance to antibiotics used in DDT was 54%, 65%, 43% and 74% respectively, to aminoglycosides 75–80%. Susceptibility to carbapenems was 94%, moxalactam 85%, ciprofloxacin 84%, piperacillin tazobactam 75%.

Conclusion: DDT as a part of the routine susceptibility tests is sufficient for ESBL screening; E-test is 20 times more expensive than DDT.